

The Relationship Between Enhanced Reticulospinal Outflow and Upper Limb Function in Chronic Stroke Patients

Authors and affiliation:

Supriyo Choudhury¹, A Shobhana¹, Ravi Singh¹, Dwaipayan Sen¹, Sidharth Shankar Anand¹, Shantanu Shubham¹, Mark R. Baker^{2,3,4}, Hrishikesh Kumar¹, Stuart N. Baker^{3,4}

1. Department of Neurology and RGC Research Centre, Institute of Neurosciences, Kolkata

2. Department of Neurology, Royal Victoria Infirmary, Queen Victoria Rd, Newcastle upon Tyne, NE1 4LP, United Kingdom

3. Department of Clinical Neurophysiology, Royal Victoria Infirmary, Queen Victoria Rd, Newcastle upon Tyne, NE1 4LP, United Kingdom

4. Institute of Neurosciences, The Medical School, Newcastle University, Framlington Place, Newcastle upon Tyne, NE2 4HH, United Kingdom

a) Name, address, telephone and email address of the **corresponding author**;

Prof. Stuart N Baker

Address:

Institute of Neuroscience

Henry Wellcome Building

The Medical School

Framlington Place

Newcastle University

Newcastle upon Tyne

NE2 4HH

Email: stuart.baker@ncl.ac.uk

Telephone: +44 (0) 191 208 8206

Fax: +44 (0) 191 208 5227

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f) Author Contribution:

Supriyo Choudhury: Study concept and design, acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content
choudhurydrsupriyo@gmail.com

A Shobhana: Patient diagnosis, critical revision of the manuscript for important intellectual content
docshobhana@yahoo.com

Ravi Singh: acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content
ravisingh.timt@gmail.com

Dwaipayan Sen: Patient diagnosis, critical revision of the manuscript for important intellectual content
dwai_sen@gmail.com

Sidharth Shankar Anand: Patient diagnosis, critical revision of the manuscript for important intellectual content
drsidharthsa@gmail.com

Shantanu Shubham: Patient diagnosis, critical revision of the manuscript for important intellectual content
shantanuj619@gmail.com

Mark R. Baker: Study concept and design, acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content
Mark.Baker@newcastle.ac.uk

Hrishikesh Kumar: Study concept and design, acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content, study supervision
rishi_medicine@yahoo.com

Stuart Baker: Study concept and design, acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content, study supervision
stuart.baker@newcastle.ac.uk

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Abstract

Background. Recent evidence from both monkey and human studies suggests that the reticulospinal tract may contribute to recovery of arm and hand function after stroke. In this study, we evaluated a marker of reticulospinal output in stroke survivors with varying degrees of motor recovery.

Methods. We recruited 95 consecutive stroke patients presenting 6 months to 12 years after their index stroke, and 19 healthy control subjects. Subjects were asked to respond to a light flash with a rapid wrist flexion; at random, the flash was paired with either a quiet or loud (startling) sound. The mean difference in electromyogram response time after flash with quiet sound compared to flash with loud sound measured the StartReact effect. Upper limb function was assessed by the Action Research Arm Test (ARAT), spasticity was graded using the Modified Ashworth Scale (MAS) and active wrist angular movement using an electrogoniometer.

Results. StartReact was significantly larger in stroke patients than healthy participants (78.4 vs 45.0 ms, $P < 0.005$). StartReact showed a significant negative correlation with the ARAT score and degree of active wrist movement. The StartReact effect was significantly larger in patients with higher spasticity scores.

Conclusion. We speculate that in some patients with severe damage to their corticospinal tract, recovery led to strengthening of reticulospinal connections and an enhanced StartReact effect, but this did not occur for patients with milder impairment who could use surviving corticospinal connections to mediate recovery.

Introduction

After suffering a stroke, individuals pass through defined stages of recovery of upper limb function^{1,2}, with early spasticity and synergistic co-activation yielding to more fractionated forms of muscle activation. A single patient may fail to progress beyond a given recovery phase, leaving them with residual disability. The pattern of recovery suggests contributions from two underlying systems³, one contributing mainly strength, and the other most important for dexterity. Given recent evidence for a role of the reticulospinal tract in recovery of hand function^{4,5}, these systems may map onto reticulospinal and corticospinal tracts respectively⁶. In stroke patients with little residual corticospinal tract, who remain heavily reliant on reticulospinal pathways even late in their recovery, the increased contribution from this pathway may lead to movement synergies^{7,8}, which limit the range of functional movements such as reaching^{9,10}.

Non-invasive approaches to assess reticulospinal function in humans are limited. One potentially useful method is the StartReact paradigm, which measures the shortening of a voluntary reaction time when a visual cue is paired with a loud sound¹¹. Given the proven role of the reticulospinal tract in overt acoustic startle responses, StartReact has been hypothesised to reflect reticulospinal activity. Increased StartReact effects are seen in patients with hereditary spastic paraplegia¹², in patients after spinal cord injury for gross but not fine grasp responses¹³, and in stroke patients¹⁴ in agreement with an enhancement of reticulospinal pathways in these conditions. In this study, we applied the StartReact method to the wrist flexor muscles of stroke patients, and correlated the degree of reaction time shortening with measures of upper limb function.

Methods

We studied 95 consecutively-recruited subjects (mean age 53 years; 67 male) who had recovered for 6 months to 12 years after a haemorrhagic or ischemic stroke, and 19 healthy controls. All subjects provided written informed consent, and procedures were approved by the Institutional Ethics Committee of the Institute of Neurosciences, Kolkata (reference number I-NK/EthicsComm/46/2016 dated 2nd April 2016). Stroke patients with brainstem involvement assessed at stroke onset by MRI, or those with visual or auditory impairment or individuals who had received botulinum toxin therapy for spasticity in the preceding three months were excluded. To avoid difficulties in understanding the task requirements, patients were screened for cognitive impairment using the mini mental state examination and excluded if they scored below 18. Stroke was determined from MRI as cortical in 24% of patients, subcortical in 40%, and mixed in the remainder.

For the more affected side, upper limb function was assessed by the Action Research Arm Test (ARAT)¹⁵, and spasticity at the wrist by the Modified Ashworth Scale (MAS)¹⁶. The angular range of active wrist movement was measured using an electrogoniometer (SG 75, Biometrics Ltd, Newport, UK). All assessments were performed by the same individual (author SC), without knowledge of the StartReact results.

StartReact was assessed using surface electromyogram (EMG) recordings from electrodes placed over the forearm flexor compartment on the more affected side. Signals were amplified (30-2000Hz bandpass, gain 2K) and sampled to hard disc at 5kHz for offline analysis (1401 interface with Spike2 software, Cambridge Electronic Design, Cambridge, UK). Subjects sat with the affected arm flexed $\sim 90^\circ$ at the elbow with the forearm in a cushioned plastic cast fixed to a table. The hand was held semi-prone with fingers extended and restrained between two vertical plastic blocks, whose spacing could be adjusted to clamp the hand firmly but comfortably in position. This arrangement was clamped to a strain gauge

suitable for isometric wrist flexion. Subjects were instructed to attend to a red light emitting diode (LED) placed ~1m in front of them, and to make a rapid wrist isometric flexion movement after it illuminated (LED on time 50ms). At random, the LED flash was delivered alone, or paired with a quiet (80dB; 500Hz, 50ms) or loud acoustic stimulus (110dB). These intensities are comparable to those which have been used in previous studies^{12,17}. The loud sound evoked a clear startle in the subjects on initial presentation. Before the start of recordings, subjects were familiarized with the task by practicing 3-4 responses to the LED flash. Five consecutive loud sounds were then delivered without task performance, to habituate the overt startle reaction. Both practice and habituation trials were discarded without analysis. Twenty responses in each condition, in randomized order, were then recorded with a 5s inter-trial interval (see Fig. 1AB for example single trial data).

The reaction time from stimulus to the first increase in EMG activity above baseline (defined as crossing pre-stimulus baseline plus three standard deviations, see Fig. 1CD) was measured on each trial automatically using a custom Matlab script, which also displayed sweeps and allowed erroneously assigned times (caused, for example, by electrical noise artefacts) to be manually corrected or excluded. We designated measures after LED flash alone the visual reaction time (VRT); when paired with quiet sound the visual-auditory reaction time (VART), and with the loud sound the visual-startle reaction time (VSRT).

In a few stroke patients, we repeated these measurements with a wrist extension movement in response to the cue, measuring reaction time from EMG collected from the forearm extensor. Such measurements were not possible in the majority of patients, due to extensor weakness, and are reported only briefly at the end of the Results for completeness.

Results

The demographic and disease profile of 95 stroke patients are presented in Table 1. Out of 95 stroke patients, useable reaction time data was obtained from 46. In the remainder, reliable measures could not be made because either they complained of fatigue after a few trials and could not complete the task or their wrist flexor EMG signal was too weak to be usable. The mean ARAT score for the patients who could perform the task successfully was, as expected, higher (22.5, SD 21.4) compared to those who failed to produce usable StartReact data (8.6, SD 9.8).

Figure 1EF illustrates results from two patients with very different residual impairment, as cumulative distribution plots of single trial measures of VRT, VART and VSRT. Patient 23 (Fig. 1E) had severely impaired upper limb function (ARAT 4/57), and there was a wide separation between the curves for VART and VSRT (mean difference 170 ms). By contrast, patient 34 (ARAT 56/57; Fig. 1F) had smaller separation between these curves, and the mean VART-VSRT difference was only 43 ms, within the range seen in healthy subjects.

Figure 2A presents the comparison of reaction time measures between the healthy participants and stroke survivors. Unsurprisingly, all reaction times were significantly prolonged in the stroke subjects. We used the difference between VART and VSRT as an estimate of the StartReact effect. This was also greater on average in the stroke subjects (all comparisons $P < 0.005$; t-test with Benjamini-Hochberg correction for multiple comparisons). This result was unaltered when we limited the comparison to a subset of stroke survivors matched to the age of the healthy participants (average age 35 years in healthy and 38 years in stroke, ages not significantly different, $P = 0.399$; Fig. 2B). Through analysis of covariance, we found that stroke was a significant predictor of reaction times and the StartReact effect even after adjusting for age as covariate ($P < 0.05$).

Although on average stroke subjects had a significantly greater StartReact effect, there was considerable heterogeneity between individuals, as illustrated already by the two subjects shown in Fig. 1. To confirm the impression that patients with greater functional impairment had a larger StartReact effect, we measured the correlation between StartReact (i.e. VART-VSRT difference) and measures of upper limb function. Scatter plots of StartReact effect versus the overall ARAT score, and active angular range of motion are shown in Fig. 3A and 3B respectively; in each case, the correlation was significant. The sub-domains of the ARAT score, and the degree of wrist flexion, were also significantly correlated with StartReact (see Table 2). Although these correlations indicate an association between the functional measures and StartReact, this is clearly not well represented by a linear relationship. For the plots in Fig. 3, it appears that patients fell into two categories: those with StartReact in the normal range (indicated by the bar underneath the abscissa) had highly variable functional scores (ARAT, Fig. 3A: mean 27.1, SD 21.5; active range of motion, Fig. 3B: mean 61.7°, SD 40.1°; n=37). Those with a StartReact effect greater than the normal range had reduced, and less variable functional scores (ARAT: mean 4.3, SD 3.6; active range of motion: mean 22.9°, SD 12.8°; n=9). These differences were significant (difference in mean, unpaired t-test, $P=0.0001$ for both ARAT and range of motion; difference in variance, Levene's test for equality of variance, both $P=0.0001$).

One possible explanation for the increased StartReact (probably reflecting an increase in reticulospinal outputs) in patients with more limited hand/wrist function is that movement was more impaired by spasticity in these individuals. The pathogenesis of spasticity is thought to involve the reticulospinal tract¹⁸. To explore this, patients were separated into mild (MAS 0-1; n=15) and moderate/severe (MAS 1.5-4; n=31) levels of spasticity. There was a significantly larger StartReact effect in the patients with greater spasticity ($P<0.05$, t-test; Fig. 4).

In addition to measuring StartReact with a wrist flexion response, we also attempted to repeat the measurement using a wrist extension response and recording EMG from the forearm extensor muscles. This proved impossible in almost all stroke survivors, due to the profound extensor weakness commonly seen after stroke. However, in 13 patients usable data were obtained. These patients were among the least impaired in our sample (ARAT mean 43.4 SD 17.0, range 9-57). There was no significant difference between Startreact measures made from the flexor and extensor muscles in these subjects (VSRT-VART for extensors mean 34.0 ms SD 29.3 ms, for flexors mean 52.3 ms SD 34.0 ms; $P>0.05$, paired t-test).

Discussion

Several previous reports have investigated reticulospinal contributions to stroke recovery using overt startle responses in more proximal muscles. Startle is enhanced in stroke patients compared to controls¹⁹, and correlates positively with spasticity^{19,20} and negatively with strength in the biceps muscle¹⁹. Within a single patient, the startle response in biceps is elicited more frequently on the more affected side²⁰. Stroke patients show slowed voluntary reaction times in the biceps and triceps muscles compared with healthy subjects, but reaction times following startling cues are similar to healthy people; this suggests that the StartReact effect for elbow muscles is greater in stroke survivors. Additionally, stroke survivors attempting an elbow extension movement generate excess and inappropriate elbow flexor activity when the movement is triggered by a startling cue; this suggests that an overt startle reflex may overlie and interfere with startle-triggered movements in these individuals²¹.

Recent work in monkey has demonstrated a role for the reticulospinal tract in hand movements as well as grosser movements involving upper arm muscles²²⁻²⁴, and that these connections can strengthen during functional recovery⁴. Consistent with this, the StartReact effect can be seen in hand muscles^{25,26}. Our finding of enhanced StartReact in stroke patients

for a wrist flexion movement, and a correlation with spasticity, is consistent with the previous studies.

How should we interpret the negative correlation between measures of upper limb function and StartReact? The obvious conclusion might be that enhancement of reticulospinal function is detrimental, and produces deficits in upper limb function. Such a conclusion may be supported by the positive correlation with spasticity (Fig. 4). Previous work has suggested that movement synergies, which often impair movements in stroke survivors⁹, have an origin in the strengthening of reticulospinal outflow after corticospinal damage²⁷⁻²⁹. It is known that the reticulospinal tract is less able to generate fractionated patterns of independent muscle activation than the corticospinal tract^{6,30,31}. If reticulospinal connections have only detrimental effects, this would suggest that therapy to improve upper limb function should concentrate on reducing reticulospinal drive.

It is important to remember, however, that the demonstration of an association between reticulospinal enhancement and poor upper limb function does not tell us anything about cause and effect. An alternative explanation is possible, based on the idea of separate systems to produce recovery of strength and dexterity, putatively linked to the reticulospinal and corticospinal tracts respectively³. On this view, where cortical damage is slight, recovery can proceed using spared corticospinal fibres from the primary motor cortex adjacent to the infarct³², or (less effectively) from non-primary motor areas^{33,34}. Such patients would be expected to recover considerable dexterity, and score well on the ARAT. There would be no need for enhanced reticulospinal connections. By contrast, where cortical damage is extensive, few spared corticospinal resources will remain. Recovery must then use the ‘second best’ alternative of reticulospinal connections, which can generate only an approximation of the desired motor output⁶ leading to synergistic patterns of muscle

activation^{27-29,35}, which are nonetheless preferable to paralysis^{28,36}. Such patients would have poor dexterity, and may have spasticity generated by aberrant reticulospinal outflow. Both factors would produce poor ARAT scores. This view is in accord with other work on ipsilateral motor evoked potentials following transcranial magnetic brain stimulation, which are most likely carried by a cortico-reticulospinal route^{4,37-39}. Patients with such ipsilateral responses typically show poorer recovery⁴⁰. On this view, an intervention which strengthens reticulospinal outputs would aid recovery, but only in severely affected individuals. More moderately affected stroke survivors should instead focus on strengthening residual corticospinal output, which may allow recovery without interfering synergies. This idea is supported by a previous report which attempted to suppress the activity of the ipsilateral cortex using transcranial direct current stimulation⁴¹. Such stimulation improved upper limb control for patients with mild impairment and limited damage to the corticospinal tract assessed by magnetic resonance imaging, but led to some *loss* of limb control in those with modest to severe impairment and greater corticospinal damage. A recent clinical trial suggested that optimizing the use of the residual corticospinal tract (over the contralesional corticoreticulospinal tract) plays an important role in the improvement of reaching function in individuals with moderate to severe stroke⁴².

The correlation coefficients which we observed between measures of hand function and StartReact were modest ($\rho = -0.3$ to -0.39 , Fig. 3); this would be consistent with the correlation being mediated indirectly via a third variable (i.e. extent of corticospinal tract damage). By contrast, a previous study²⁸ measured the correlation between the Fugl-Meyer score (a measure of impairment) and a laterality index of brain activation (presumably measuring contralateral cortico-reticulospinal recruitment), and found stronger correlations ($r^2 = 0.57$ to 0.93). This would be consistent with a more direct mediation.

It is of interest that, if we consider only the most severely impaired subjects with active range of motion below 60°, there appears to be a bimodal distribution of StartReact effect (Fig. 3B). It has been previously reported that severely impaired stroke survivors fall into two groups: those who do, and do not, follow the ‘proportional recovery rule’ and recover ~70% of lost function⁴³. Given that we made our measurements at least six months after stroke, it is likely that recovery had reached a plateau. The subjects who remained severely impaired in our cohort are thus likely to be the ‘non-fitters’ to the proportional recovery rule; it is known that these individuals are likely to have the most impaired corticospinal tract⁴⁴. Our results may therefore suggest that these individuals can be further divided into those that have, and have not, substantially strengthened their reticulospinal outflow. This could be affected by many factors, most obviously by the detailed location of the lesion. In agreement with this, a previous report showed that more severely affected patients had, on average, an increased output from the ipsilateral hemisphere to the paretic limb compared with less impaired patients; however, there was considerable variability within the severely affected group⁴⁵.

In this study, we concentrated on wrist flexion movements. This is in contrast to a previous report by Honeycutt et al which examined StartReact during wrist extension and found no difference between stroke survivors and healthy controls⁴⁶. However, wrist extension is frequently impaired after stroke, with many stroke survivors unable to make an extension effort. Measurement of StartReact in wrist extension necessarily therefore involves recruitment of less impaired individuals; in agreement with this, in Honeycutt et al’s work 7/8 subjects had Chedoke hand scores ≥ 4 (out of a maximum of 7); the single subject with a Chedoke score of 3 exhibited no StartReact effect. Our own limited dataset from a few patients capable of wrist extension also found no difference in StartReact between flexion and extension responses. Reticulospinal connections to forearm extensors do not seem to

strengthen after corticospinal lesions⁴⁷, which may explain the lack of extensor function in individuals unable to recruit sufficient spared corticospinal fibres to the recovery process.

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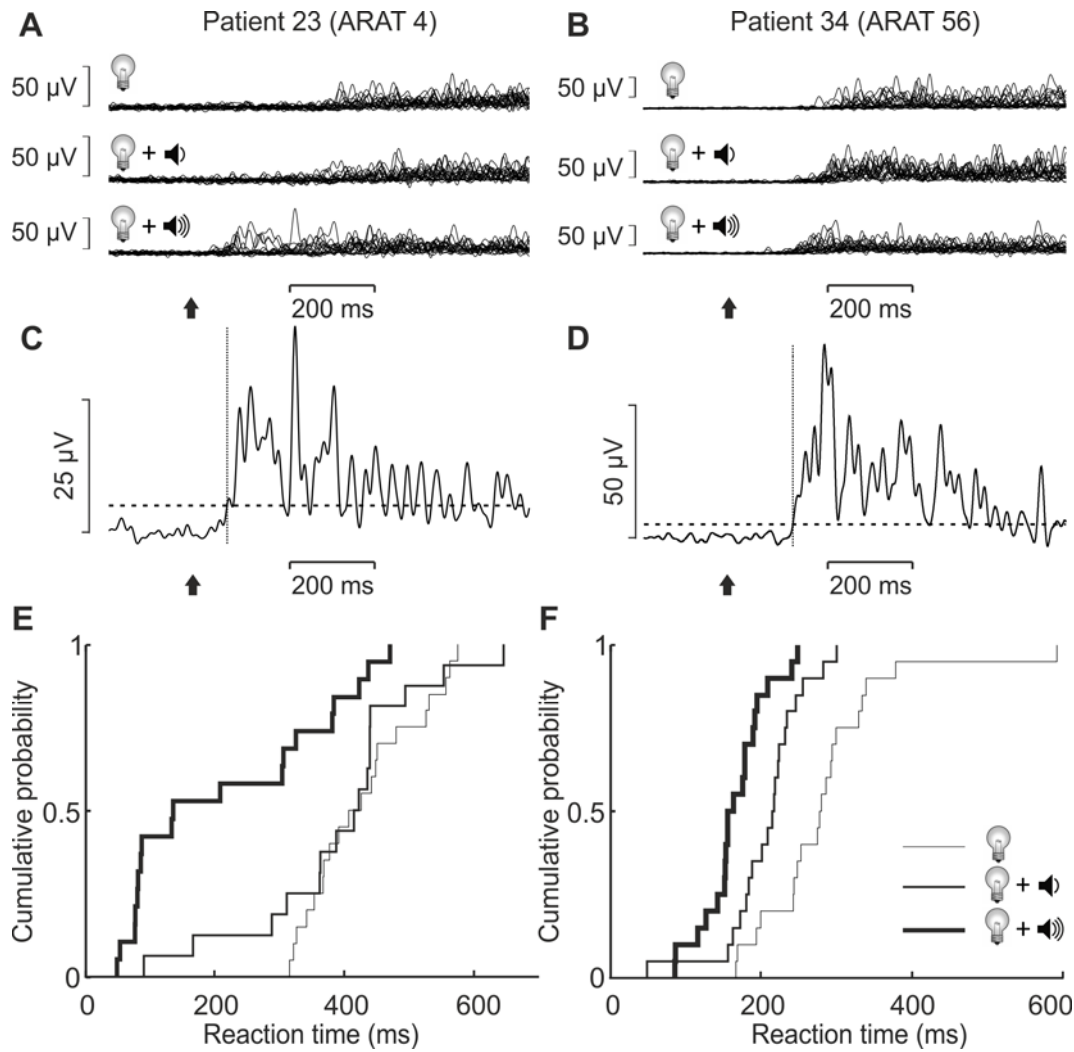


Figure 1. Representative data from two patients. Left column shows data from patient 23 who had severely impaired upper limb function, with an Action Research Arm Test (ARAT) score of 4/57; right column from patient 34 who had minimal impairment (ARAT 56/57). A, B, EMG recordings from the forearm flexors. Recordings have been rectified and low-pass filtered; trials are overlain for the three tested conditions of visual reaction time (VRT, light flash cue only), visual-auditory reaction time (VART, light flash plus quiet sound cue) and visual-startle reaction time (VSRT, light flash plus loud sound cue). C, D, single trial EMG responses, indicating the threshold set to the mean plus 3SD of the baseline (horizontal dashed line), and the detected reaction time (vertical dotted line). Arrows indicate cue onset time. E, F, cumulative distribution of detected reaction times in the three conditions. Note the greater separation between VART (medium line) and VSRT (thick line) in (E) than in (F).

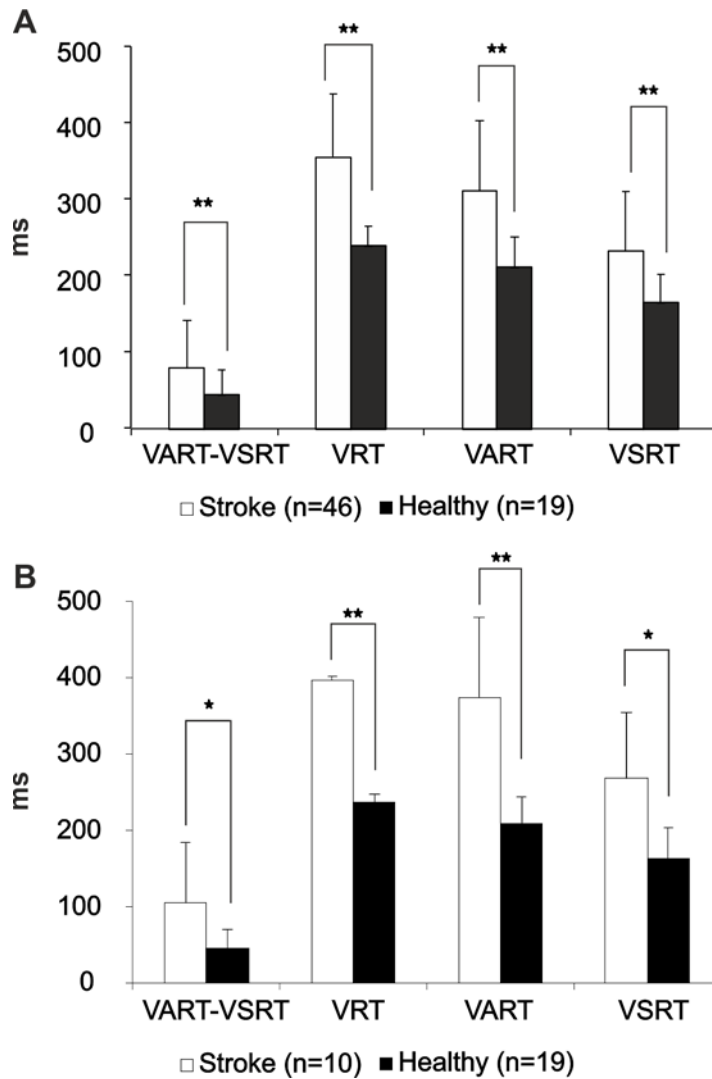


Figure 2. Comparison of StartReact responses between healthy subjects and stroke survivors. A, comparison between all 46 stroke survivors in which VSRT-VART could be measured, and 19 healthy participants. B, comparison between 19 healthy participants and 10 age-matched stroke survivors. In both cases, stroke survivors demonstrated a significantly larger StartReact effect (VART-VSRT) compared to healthy participants (unpaired t-test). They also showed early visual reaction times (VRT), visual auditory reaction times (VART) and visual startle reaction times (VSRT). Error bars plot SD. The normality of the data were tested by one sample KS Test ($p > 0.05$). The homogeneity of variance was assessed by Levene's test for equality of variance and accordingly the type of unpaired t-test was selected. P value of unpaired t-test is indicated by **, $P < 0.005$; *, $P < 0.05$, corrected for multiple comparisons by the Benjamini-Hochberg procedure.

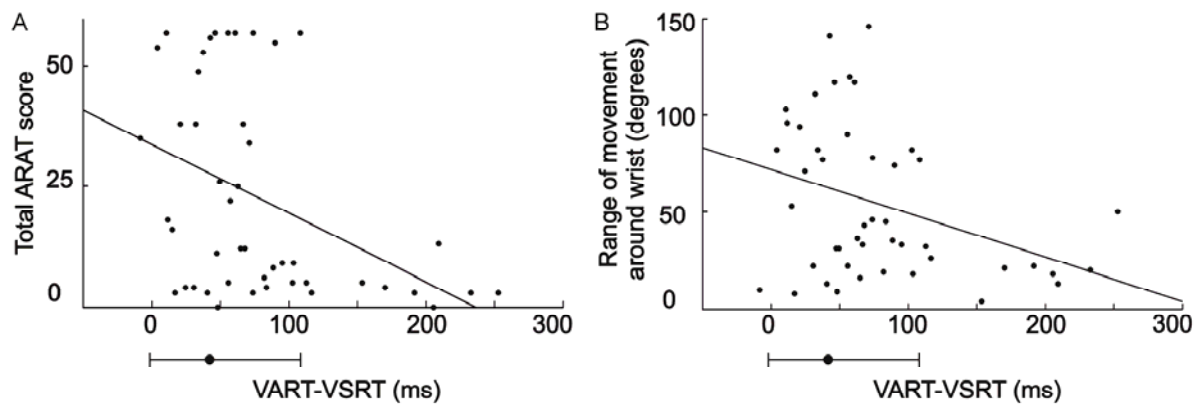


Figure 3. Scatterplots showing correlation between startle response and measures of upper limb function in stroke survivors. Scatterplots summarising the correlation between StartReact (VART-VSRT) and A. ARAT score (maximum 57), B. Active angular range of movement around the wrist. Note the significant negative correlation between VART-VSRT and total ARAT score ($\rho = -0.393$; $p = 0.007$) and VART-VSRT and active angular wrist movement ($\rho = -0.303$; $p = 0.043$). The horizontal error bar below the abscissa indicates the median and 95% range of VART-VSRT in healthy individuals. ARAT, Action Research Arm Test; VART, Visual auditory response time; VSRT, Visual startle reaction time. Results are presented for 46 patients in whom VART-VSRT could be measured.

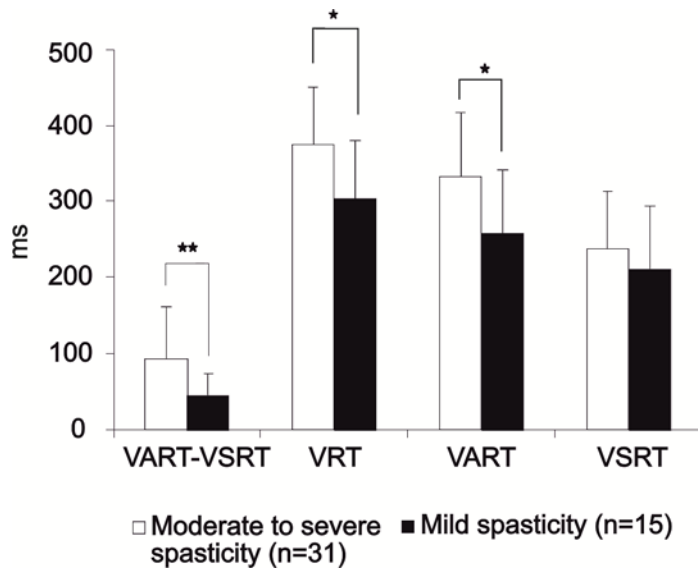


Figure 4. Comparison of StartReact responses in stroke patients grouped according to severity of spasticity. Bar chart comparing the effect of spasticity on various reaction time parameters. Patients with higher spasticity (Modified Ashworth score >1) showed significantly larger StartReact measure (VSRT-VART) compared to patients with mild upper limb spasticity (Modified Ashworth score 0-1) according to the unpaired t-test (p values are included in the bar chart). Error bars plot SD. VRT, Visual response time; VART, Visual auditory response time; VSRT, Visual startle reaction time. Data were assessed as normally distributed (KS Test, $P > 0.05$). The homogeneity of variance was assessed Levene's test for equality of variance and the type of unpaired t-test was selected accordingly. P value of unpaired t-test indicated by: **, $P < 0.005$; *, $P < 0.05$, corrected for multiple comparisons by the Benjamini-Hochberg procedure. Results are presented for 46 patients in whom VART-VSRT could be measured.